Genetic Resources and Crop Evolution (2005) 52: 601–607
DOI 10.1007/s10722-004-0583-z

Genetic analysis of Egyptian date (*Phoenix dactylifera* L.) accessions using AFLP markers

Ashraf M. El-Assar, Robert R. Krueger[1], Pachanoor S. Devanand[2] and Chih-Cheng T. Chao*
Tropical Fruit and Date Palm Research Department, Horticultural Research Institute, 9 Cairo University Street, Orman, Giza, Egypt; [1]*National Clonal Germplasm Repository for Citrus and Dates, USDA-ARS, 1060 Martin Luther King Boulevard, Riverside, CA 92507, USA;* [2]*Department of Botany and Plant Sciences, University of California-Riverside, Riverside, CA 92521, USA; *Author for correspondence (e-mail: ctchao@citrus.ucr.edu; phone: 01-909-787-3441; fax: 01-909-787-4437)*

Received 19 November 2003; accepted in revised form 19 March 2004

Key words: Genetic similarity, Germplasm, Molecular markers, *Phoenix dactylifera*

Abstract

Forty-seven samples of date palm (*Phoenix dactylifera* L.) collected from eight locations in Egypt were studied using four sets of amplified fragment length polymorphism (AFLP) markers with near infrared fluorescence labeled primers. These samples belonged to 21 named accessions and 9 of unknown pedigrees. A total of 350 bands were scored and 233 (66.6%) were polymorphic. Twenty-seven Egyptian accessions and 'Medjool' and 'Deglet Noor' accessions from California could beclassified into the major cluster. This major cluster may represent a major group of date palm germplasm in North Africa. There were four other clusters, each containing one or two accessions. The variety 'Halawy' and one accession of unknown provenance were most likely from hybridization between two clusters. Six groups of accessions of which had the same names, revealed similar but not identical AFLP profiles suggesting these accessions might derive from seedlings rather thanthrough clonal offshoot propagation.

Abbreviations: AFLPs – amplified fragment length polymorphism; UPGMA – unweighted pair group method of the arithmetic average; PCA – principal coordinated analysis; PCR – polymerase chain reaction

Introduction

Dates (*Phoenix dactylifera* L.) ($2n = 2x = 36$) are dioecious, perennial, monocotyledonous fruit trees that belong to the family of Arecaceae (Coryphoideae) (Barrow 1998). Date is the major fruit crop of arid climate region in the countries of North Africa and the Middle East. Egypt is the leading date production country in the world. Egypt has very long history of date cultivation that dates back to 3200 BC. According to Brown (1924) and Mason (1927), the common commercial date varieties traditionally grown in Egypt were 'Amhat,' 'Barakawi,' 'Bentamoda,' 'Hayany,' 'Saidy' ('Siwi'), 'Sammany' and 'Zaghloul.' Seedlings were also commonly grown in most regions. Date varieties can be classified as dry, semi-dry and soft dates based on the fruits at harvest.

Markers such as isozyme, random amplified polymorphic DNA (RAPD), and restriction fragment length polymorphism (RFLP) have been used to identify date palm accessions (Torres and

Tisserat 1980; Baaziz and Saaidi 1988; Al-Jibouri and Adham 1990; Bennaceur et al. 1991; Corniquel and Mercier 1994, 1997; Bendiab et al. 1998; Mokhtar et al. 1998; Saker and Moursy 1998; Sedra et al. 1998; Salem et al. 2001). Soliman et al. (2003) used RAPD markers to study 3 males and 4 female date accessions from Egypt. They found the genetic similarity between the four female accessions ranging from 87.5 to 98.9%. The AFLP marker has been used in the study of a number of fruit tree species in recent years. The primers, restriction enzymes, and composition of the genomic DNA dictate the complexity of the AFLP profiles. These markers can generate large numbers of polymorphisms for both inter- and intraspecies identification. Our previous work using fluorescent-AFLP markers differentiated 21 California date cultivars (Cao and Chao 2002). We also used AFLP markers to study the intravarietal difference within the 'Medjool' date accessions in California (Devanand and Chao 2003). AFLP markers also were used to develop a genetic map of date palms (El-Kharbotly et al. 1998).

In the current study, we used fluorescent-labeled AFLP markers to study the genetic variation of date accessions from Egypt. We established AFLP profiles and identified the genetic variation within each variety.

Materials and methods

Plant materials

Forty-seven samples of Egyptian date palm were collected from six locations in Egypt representing the main date-growing regions. The Damietta region is located in the Nile Delta along the Mediterranean Sea. The Rasheid region is located in the West of Nile Delta on the Mediterranean coast. The Ismailia region is North-East of Cairo next to the Suez Canal. The Assiut region is located in the mid-Nile Valley. The Aswan region is located in the Southern end of the Nile Valley and Nasser lake. The Faiyum regions is located in the South-West of Giza in the Egyptian western desert, around the Qaroun lake. The Siwa Oasis is located west of the Nile Valley in the western desert. In addition, two date accessions from the United States Department of Agriculture, National Clonal Germplasm Repository for Citrus and Dates at the University of California-Riverside, Coachella Valley Agricultural Research Station (CVARS) at Thermal, California were used in the study. Their names, countries and locations of collection, countries of origin, and additional information on all materials used in the study are listed in Table 1.

DNA isolation and fluorescent-AFLP analysis

Total DNA was extracted from young leaves of the date palms using the DNeasy system (Qiagen, Valencia, CA, USA). DNA concentrations were quantified using a Hoefer DyNA Quant 200 (Pharmacia Biotech, Piscataway, NJ, USA). AFLP analysis was conducted using the GIBCO BRL AFLP System II (Life Tecnologies, Grand Island, NY, USA) and visualized with the Li-COR 4000-L IR automated sequencer (Li-COR Inc., Lincoln, Neb., USA). Total DNA (125 ng) from all samples was digested with 1 μL of mixture of *Eco*RI/*Mse*I (1.25 units/μL) at 37 °C overnight, and ligated to *Eco*RI/*Mse*I adapters with 1.5 μL (1 unit/μL) of T4 DNA ligase at 25 °C for at least 6 h. The adaptor-ligated DNA was amplified using a mixture of 2.5 μL of DNA from the ligation reaction, 20 μL of Pre-amp mix II, 2.5 μL of 10× PCR buffer, and 0.2 μL of *Taq* DNA polymerase (5 units/μL). The pre-amplification reactions were performed on a MJR Cycle LR^{TM} (MJ Research, Inc., Watertown, MA, USA) using the following cycling parameters: 30 cycles at 94 °C for 15 s, 56 °C for 30 s + 1 s/cycle, and 72 °C for 1 min + 1 s/cycle, then 1 cycle at 72 °C for 3 min. The quantity of the DNA in the pre-amplified PCR product was checked in a flurometer, and the amount of template for subsequent PCR was adjusted by dilution. Selective amplification was performed using a diluted pre-amplification product with near infrared fluorescence dye (IRD) labeled *Eco*RI primers: 2 μL of DNA from pre-amplification, 2 μL of *Mse*I primer, 0.5 μL of IRD700-labeled *Eco*RI primer, 0.5 μL of IRD800-labeled *Eco*RI primer, 1 μL of 10× PCR buffer, 4 μL of H_2O, and 0.16 μL of *Taq* DNA polymerase (5 units/μL). The selective amplification PCRs were performed by another touchdown program as follows: 13 cycles at 94 °C for 15 s, 65 °C for 30 s − 0.7 °C/cycle, and 72 °C for 1 min, then 30 cycles at 94 °C for 15 s, 56 °C for 30 s + 1 s/cycle, 72 °C for 1 min + 1 s/cycle, then 72 °C for 3 min. Both pre- and selective-

Table 1. Forty-seven accessions from Egypt and two accessions of date palms from USA used in the study.

No.	Accessions	Collection location	Collection countries	Synonym	Country of origin	Note
1	Agglany	Ismailia	Egypt		Egypt	
2	Amhat	Siwa	Egypt			
3	Ammry 1	Assiut	Egypt	Ammary	Egypt	
4	Ammry 2	Ismailia	Egypt	Ammary	Egypt	
5	Baldy unknown	Aswan	Egypt		Egypt	
6	Bartmouda 1	Aswan	Egypt		Egypt	
7	Bartmouda 2	Aswan	Egypt		Egypt	Tissue culture
8	Bint Aisha 1	Assiut	Egypt		Egypt	
9	Bint Aisha 2	Damietta	Egypt		Egypt	
10	Bint Aisha 3	Ismailia	Egypt		Egypt	
11	Bint Aisha 4	Rasheid	Egypt		Egypt	
12	Chammeia	Aswan	Egypt		Egypt	
13	Deglet Noor	Thermal	USA		Algeria/Tunisia	
14	Degna	Aswan	Egypt		Egypt/Sudan	
15	Feryhy	Siwa	Egypt		Egypt	
16	Gargouda	Aswan	Egypt		Egypt/Sudan	
17	Gondailah	Aswan	Egypt	Gondeila	Egypt/Sudan	
18	Halawy	Assiut	Egypt		Egypt	
19	Hayany 1	Assiut	Egypt		Egypt	
20	Hayany 2	Damietta	Egypt		Egypt	
21	Hayany 3	Ismailia	Egypt		Egypt	
22	Hayany 4	Rasheid	Egypt		Egypt	
23	Hommera unknown	Aswan	Egypt		Egypt	
24	Malakaby	Aswan	Egypt		Egypt	
25	Medjool	Thermal	USA		Morocco	
26	Nasser Dein	Rasheid	Egypt		Egypt	
27	Oreibby	Damietta	Egypt		Egypt	
28	Red unknown	Damietta	Egypt		Egypt	
29	Sakkoutty 1	Aswan	Egypt		Egypt/Sudan	
30	Sakkoutty 2	Aswan	Egypt		Egypt/Sudan	Tissue culture
31	Sammany 1	Assiut	Egypt	Samany	Egypt	
32	Sammany 2	Faiyum	Egypt	Samany	Egypt	
33	Sammany 3	Ismailia	Egypt	Samany	Egypt	
34	Sammany 4	Rasheid	Egypt	Samany	Egypt	
35	Shakngobil	Siwa	Egypt		Egypt	
36	Siwi 1	Assiut	Egypt		Egypt	
37	Siwi 2	Faiyum	Egypt		Egypt	
38	Siwi 3	Siwa	Egypt		Egypt	
39	Taktakt	Siwa	Egypt		Egypt	
40	Unknown 1	Ismailia	Egypt		Egypt	
41	Unknown 2	Rasheid	Egypt		Egypt	
42	Unknown 3 (Aeinat)	Aswan	Egypt		Egypt	
43	Unknown 4 (Aggwa)	Aswan	Egypt		Egypt	
44	Yellow Balady unknown	Faiyum	Egypt		Egypt	
45	Yellow unknown	Damietta	Egypt		Egypt	
46	Zaghloul 1	Assiut	Egypt	Zagloul	Egypt	
47	Zaghloul 2	Damietta	Egypt	Zagloul	Egypt	
48	Zaghloul 3	Rasheid	Egypt	Zagloul	Egypt	
49	Zahgloul 4	Ismailia	Egypt	Zagloul	Egypt	

amplification conditions were modified according to Myburg et al. (2000). The products from the selective amplification were electrophoresed on 25 cm × 0.25 mm of 8% denaturing polyacrylamide Long Ranger® gel solution (BMA, Rockland, Maine, USA) in 0.8× TBE buffer using a Li-COR 4000-L automated sequencer. The gel was pre-run for 30 min at 1500 V, 40 mA, and 40 W until the gel temperature reached 50 °C. The samples were denatured at 95 °C for 3 min and immediately placed on ice. We load 1.15 μL samples and 1 μL mixture of IRD700 and IRD800 size markers

(Li-COR Inc., Lincoln, Neb., USA). Samples were electrophoresed at 1500 V, 50 °C for 3.5 h. Based on our previous study (Cao and Chao 2002; Devanand and Chao 2003), we selected 4 primer sets for this study: IRD700 E + TA/M + CAG, IRD800 E + AC/M + CAG, IRD700 E + TG/M + CAT, and IRD800 E + AG/M + CAT.

Data analysis

For the genetic similarity analysis, AFLP bands were visually scored as present (1) or absent (0) to create the binary data set. These data were entered into a binary data matrix as discrete variables. Jaccard's coefficient of similarity (Sneath and Sokal 1973) was calculated for all pair-wise comparisons among the 47 Egyptian date accessions and two California date accessions as follows: Jaccard $= N_{AB}/(N_{AB} + N_A + N_B)$, where N_{AB} is the number of bands shared by two accessions (A and B), N_A represents amplified fragments in accession A and N_B represents fragments in accession B. A dendrogram was generated by cluster analysis using the unweighted pair group method of the arithmetic averages (UPGMA). Principal coordinated analysis (PCA) was also carried out to show multiple dimension of the distribution of the accessions in a scatter-pot (NTSYS-pc, version 2.1) (Rohlf 2000).

Results and discussion

AFLP markers and polymorphism

Four primer sets previously used in the studies of California date accessions were applied to the date accessions from Egypt. A total of 350 bands were generated using these four primer sets. On average, each primer generated 87.5 bands, slightly higher than the average bands (82) detected with California date accessions. There were 233 polymorphic bands (66.6%) among the Egyptian date palms. Sedra et al. (1998) used 19 random amplified polymorphic DNA (RAPD) primers to study 43 Moroccan date accessions. In total, 56 RAPD bands were generated and 37 bands were polymorphic (66.1%). The levels of polymorphism between the two marker systems were very similar.

Genetic diversity among Egyptian date accessions

The dendrogram of 47 Egyptian and two California date accessions resulting from an UPGMA cluster analysis based on Jaccard's estimates of similarity was obtained based on 350 AFLP bands (data not shown). The scatter diagram of first two principal coordinates (PC1 and PC2) from PCA analysis of 47 Egyptian and two California date accessions based on 350 AFLP bands is shown in Figure 1. The UPGMA and PCA analyses suggested that there were 5 clusters of accessions among the Egyptian date accessions: group 1 included 27 Egyptian accessions, and 'Medjool,' and 'Deglet Noor' from California; group 2 contained 'Siwi' only; group 3 consisted of the varieties 'Sammany' and 'Zaghloul'; groups 4 had only 'Hayany'; and group 5 comprised three 'Bint Aisha' accessions. Variety 'Halawy' and accession 'Unknown 2' were positioned between two or multiple groups, possibly suggesting hybrid origins for these two accessions or they may represent different germplasm resources. This Egyptian 'Halawy' has reddish fruit that is different from 'Halawy' of Iraq that has yellowish fruit color.

The 27 Egyptian accessions plus 'Deglet Noor' and 'Medjool' of cluster 1 are the largest group found among all accessions. This group of accessions may represent the major natural group of date germplasm in the North Africa region. Most of the accessions in this group may have similar origins and it will be interesting to compare this group of accessions with accessions from the ancient Mesopotamia region. The variety 'Siwi' of cluster 2 is similar to 'Sammany' and 'Zaghloul' that they are all soft dates eaten in an unripe state. 'Siwi' was grown exclusively in the ancient Giza Province in the early 1900 s (Brown 1924). The cluster 3 varieties 'Sammany' and 'Zaghloul' were two of the most commonly grown date accessions in Egypt in the early 1900s. These two varieties mature about the same time (mid September to mid October). 'Sammany' was grown exclusively in the Rosetta and Idku districts in the early 1900s (Brown 1924). In the previous study of date accessions from California (Cao and Chao 2002), 21 date varieties were separated into four groups. Group I included 10 varieties. Group II consisted of 'Halawy,' 'Medjool' and 5 other varieties. Group III encompassed 'Deglet Noor' and

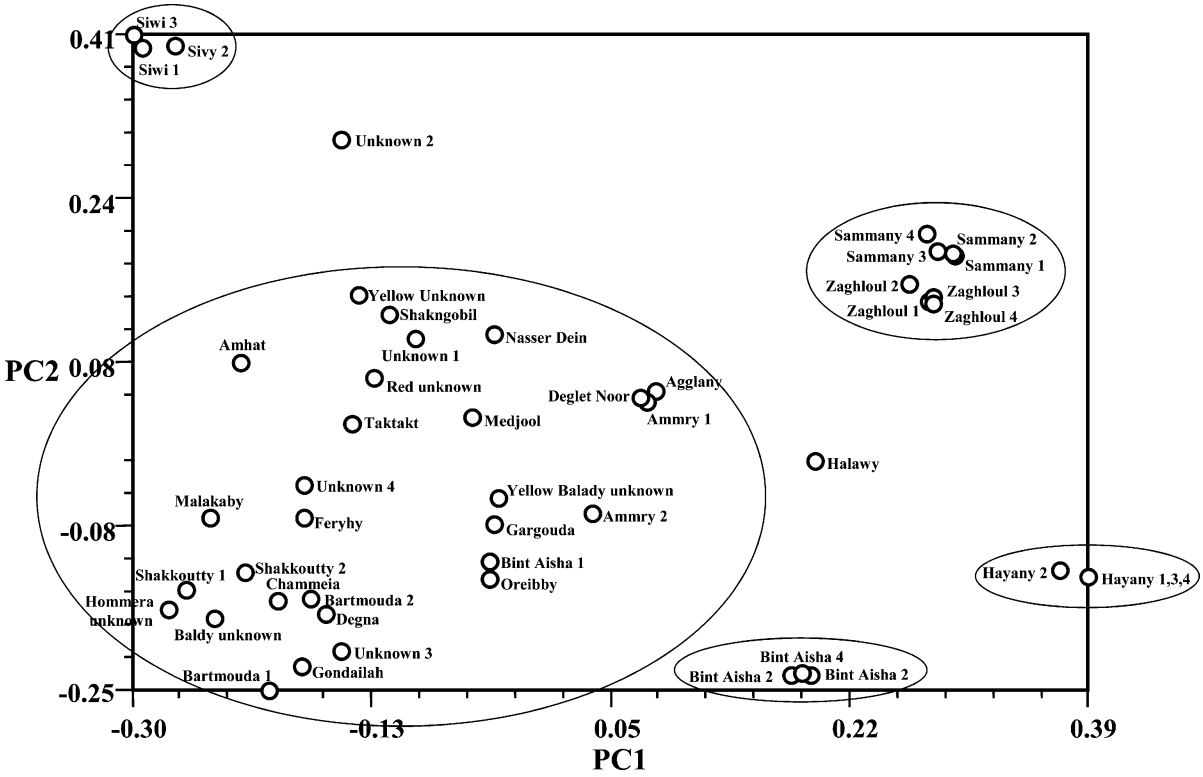

Figure 1. Scatter diagram of first two principal coordinates (PC1 and PC2) from PCA of 47 Egyptian and two California date accessions based on 350 AFLP bands.

'Barhee.' Group IV contained 'Hayany.' 'Hayany' in California had the lowest genetic similarity comparing with 20 varieties in that study. Similar results were found in current study of Egyptian accessions. The Egyptian 'Hayany' of cluster 4 was distant from all other Egyptian accessions (Figure 1). These results suggest that variety 'Hayany' could be an unique germplasm source that is different from other clusters. The cluster 5 'Bint Aisha' accessions are semi-dry dates. This variety was mainly cultivated in the northern and eastern parts of the Nile Delta in early 1900s (Brown and Bahgat 1938).

Genetic differences among accessions of the same names

Eight of the varieties studied ('Ammry', 'Bartmouda,' 'Bint Aisha,' 'Hayany,' 'Sakkoutty,' 'Sammany,' 'Siwi,' and 'Zaghloul') had multiple accessions either collected from different locations in Egypt or from tissue culture sources (Table 1). Three accessions of 'Hayany' had the same AFLP profile and the fourth 'Hayany' accession had near identical similarity (95%). Four other groups of accessions also showed similar but not identical AFLP profiles. Jaccard's genetic similarities ranged from 91–94% for three 'Bint Aisha' accessions, 93–97% for all 'Sammany' accessions, 86–91% for all 'Siwi' accessions, and 93–94% for all 'Zaghloul' accessions. These results suggest that these accessions of the same names collected from different locations may derive from sexual hybridization instead by clonal (offshoot) propagation. It is very common in North Africa and the Arabian Peninsula that sexual seedlings (commonly known as 'khalt' or 'balady') are used in commercial propagation and production.

There was one accession each of 'Bartmouda' and 'Sakkoutty' originating from tissue culture (Table 1). Both of these two tissue culture derived accessions had similar but not identical AFLP profiles with the other accessions of the same variety (75% similarity for two 'Bartmouda' and 77% for two 'Sakkoutty'). Tissue culture

propagation has been used extensively by the date industries of Egypt, Morocco, Saudi Arabia, United Arab Emirates, and other countries (Sharma et al. 1984; El Hadrami et al. 1995). However, high levels of somaclonal variation from date tissue culture are known to exist and can be detected by morphological differences and isozyme polymorphism (Azeqour et al. 2002) or by RAPD markers (Letouze et al. 1998).

One accession of 'Bint Aisha' ('Bent Aisha 1') showed large AFLP polymorphisms compared to the three other 'Bint Aisha' accessions (67, 63, and 64%, respectively). Due to the long history of cultivation of date palms in North Africa and extensive and complicated exchanges of germplasm from one location to the other, and the abundant presence of synonyms and homonyms existing in date palm accessions (Nixon 1950). 'Bint Aisha 1' may not be a true 'Bint Aisha.' However, it is also possible that the accession 'Bint Aisha 1' was a sexual seedling from a wide hybridization background that involved 'Bint Aisha.' The other possibility is that the existence of a landrace variety (Devanand and Chao 2003) may explain some large difference observed in some of these Egyptian date accessions. 'Bent Aisha 1' could also be a mistake in labeling and it could actually be an 'Oreibby.' Accessions 'Ammry 1' and 'Ammry 2' only shared 46% genetic similarities. These two accessions may not be the same varieties or may be from wide hybridization origins.

Implication for future date germplasm collection in Egypt

Our AFLP marker study showed that there is large genetic diversity among date germplasm in Egypt. The accessions could be separated into 5 clusters. The largest cluster may represent the major group of North Africa date palm germplasm materials. The other four clusters of Egyptian date accessions may represent different but minor germplasm sources in North Africa. It may be important in the future to collect, study, and maintain germplasm that are different from the major commercially desirable date varieties such as 'Deglet Noor.' As the market demand for 'Deglet Noor' like fruit increase, the acreages of other date accessions are disappearing (Centre de Recherche Phoenicicole de Dégache 2002). It may become critical to maintain germplasm other than the 'Deglet Noor'-type of dates represented in cluster 1. Other sources of date palm germplasm as represented by the 4 other groups, as well as uncollected germplasm, need further study. Differences and similarities with the 'Deglet Noor' group need to be clarified, and morphological and horticultural traits studied. The AFLP method provides a tool for a more thorough assessment of pertinent characteristics of date palms.

Acknowledgment

We acknowledge funding support from the US-Egypt Science and Technology Foundation BIO5-001-009.

References

Al-Jibouri A.A.M. and Adham K.M. 1990. Biochemical classification of date palm male cultivars. J. Hort. Sci 65: 725–729.

Azeqour M., Majourhat K. and Baaziz M. 2002. Morphological variations and isoenzyme polymorphism of date palm clones from *in vitro* culture acclimatized and established on soil in South Morocco. Euphytica 123: 57–66.

Baaziz M. and Saaidi M. 1988. Preliminary identification of date palm cultivars by esterase isoenzymes and peroxidse activities. Can. J. Bot. 66: 89–93.

Barrow S. 1998. A monograph of *Phoenix* L. (Palmae: Coryphoideae). Kew Bull. 53: 513–575.

Bendiab K., Baaziz M. and Majourhat K. 1998. Preliminary date palm cultivar composition of Moroccan palm groves as revealed by leaf isozyme phenotypes. Biochem. Syst. Ecol. 26: 71–82.

Bennaceur M., Lanaud C., Chevallier M.H. and Bounaga N. 1991. Genetic diversity of the date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. Plant Breeding 107: 56–69.

Brown T.W. 1924. Date palm in Egypt. Ministry Agri. Egypt, Tech. Sci. Service Bull. 43: 39.

Brown T.W. and Bahgat M. 1938. Date-palm in Egypt. Ministry Agri. Egypt, Tech. Sci. Service Booklet 24: 117.

Cao B.R. and Chao C.T. 2002. Identification of date (*Phoenix dactylifera*) cultivars in California using AFLP markers. HortScience 37: 966–968.

deRecherchePhoenicicole de Dégache. 2002. Sauvegarder la diversité génétique du palmier dattier à l'oasis de Dégache (Tunisie). Problèmes et perspectives. International Centre for development oriented Research in Agriculture (ICRA) working document No. 107, pp. 89.

Corniquel B. and Mercier L. 1994. Date palm (*Phoenix dactylifera* L.) cultivar identification by RFLP and RAPD. Plant Sci. 101: 163–172.

Corniquel B. and Mercier L. 1997. Identification of date palm (*Phoenix dactylifera* L.) cultivars by RFLP: partial characterization of a cDNA probe that contains a sequence encoding a zinc finger motif. Int. J. Plant Sci. 158: 152–156.

Devanand P.S. and Chao C.T. 2003. Genetic variation within 'Medjool' and 'Deglet Noor' date (*Phoenix dactylifera* L.) cultivars in California detected by fluorescent-AFLP markers. J. Hort. Sci. Biotech. 78: 405–409.

El Hadrami I., Cheikh R. and Baaziz M. 1995. Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. Biol. Plant. 37: 205–211.

El-Kharbotly A., El-Mardi M., Al-Saadi N. and Al-Mahruki Y. 1998. Towards the construction of a genetic map of date palms using the amplified fragment length polymorphism technique (AFLP). In: Proceedings of the First International Conference on Date Palms, Al-Ain, United Arab Emirates, pp. 194–207.

Letouze R., Daguin F., Satour P., Hamama L. and Marionnet F. 1998. Molecular characterization of Egyptian date palm: II. RAPD fingerprints. In: Proceedings of the First International Conference on Date Palms, Al-Ain, United Arab Emirates, pp. 158–167.

Mason S.C. 1927. Date culture in Egypt and the Sudan. USDA Bulletin No. 1457, Washington, DC, USA, pp. 72.

Mokhtar T., AbdelAli B., Abdelmajid R., André R. and Mohamed M. 1998. Molecular characterization of Tunisian date palm cultivars. In: Proceedings of the First International Conference on Date Palms, Al-Ain, United Arab Emirates, pp. 183–193.

Myburg A.A., O'Malley D., Sederoff R.R. and Whetten R. 2000. High-throughput multiplexed AFLP analysis of interspecific hybrids of *Eucalyptus* trees species. In: Plant and Animal Genome VIII Conference, San Diego, USA, pp. 544.

Nixon R.W. 1950. Imported cultivars of dates in the United States. USDA Circular No. 834, Washington, DC, USA, pp. 144.

Rohlf F.J. 2000. NTSYSpc, Numerical Taxonomy and Multivariate Analysis System, Version 2.1 User Guide. Exeter Software, Setauket, New York, USA.

Saker M.M. and Moursy H.A. 1998. Molecular characterization of Egyptian date palm: II. RAPD fingerprints. In: Proceedings The First International Conference on Date Palms. Al-Ain, United Arab Emirates, pp. 173–182.

Salem A.O.M., Trifi M., Sakka H., Rhouma A.and Marrakchi M. 2001. Genetic inheritance analysis of four enzymes in date palm (*Phoenix dactylifera* L.). Genet. Resour. Crop Evol. 48: 361–368.

Sedra M.H., Lashermes P., Trouslot P., Combes M. and Hamon S. 1998. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) cultivars from Morocco using RAPD markers. Euphytica 103: 75–82.

Sharma D.R., Dawra S. and Chowdhury J.B. 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* Linn.) cv. 'Khadravi' through tissue culture. Indian J. Exp. Biol. 22: 596–598.

Sneath P.H.A. and Sokal R.R. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.H. Freeman and company, San Francisco, USA, pp. 359.

Soliman S.S., Ali B.A., Ahmed and M.M.M. 2003. Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. African J. Biotech. 2: 86–87.

Torres A.M. and Tisserat B. 1980. Leaf isozyme as genetic markers in date palms. Am. J. Bot. 67: 162–167.